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The highly variable release call of the missing Northern Darwin's Frog, *Rhinoderma rufum*

[1,2,3]José M. Serrano, [1]Gabriel Bidart-Enriquez, and [1,]*Mario Penna

[1]*Programa de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Independencia 1027, 8380453, Santiago, CHILE* [2]*ONG Ranita de Darwin, Santiago, CHILE* [3]*Laboratorio de Comunicación Animal, Vicerrectoría de Investigación y Postgrado, Universidad Católica del Maule, San Miguel 3605, 3480112, Talca, CHILE*

***Abstract.*—The release calls of *Rhinoderma rufum* (Philippi 1902) are described quantitatively based on recordings of four males of this species obtained in 1981 from a population in central Chile. This record corresponds to the last scientific sighting of the species. The release calls of *R. rufum* consist of sequences of complex notes containing harmonics and non-linear phenomena, with chaos segments and highly variable acoustic properties. This characterization expands the acoustic repertoire of this endemic and likely extinct species, and contributes to differentiating it from the extant congeneric *R. darwinii*.**

Keywords. Anurans, bioacoustics, central Chile, endemic, endangered species, non-linear phenomena, vocalization

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Introduction

The release calls of anurans are signals that allow rejection of unwanted amplexus and may also have an anti-predator function (Köhler et al. 2017). In addition, empirical evidence in several anuran species suggests that release calls play an important role in sexual recognition (Aronson 1943; Bowcock et al. 2008; Liao and Lu 2009; Penna and Veloso 1981). The acoustic properties of release calls have been shown to have a phylogenetic footprint (di Tada 2001; Forti et al. 2018), and therefore the characterization of these signals contributes highly relevant knowledge for endangered species (Márquez et al. 2018; Stănescu et al. 2019).

Rhinoderma is an anuran genus endemic to the temperate forests of southern South America in Chile and Argentina, and a symbolic entity of the current global amphibian extinction crisis (Azat et al. 2021). Two species of Darwin's Frog are currently recognized: the northern species, *Rhinoderma rufum,* which is probably extinct, as the last report of living specimens dates back to 1981 (IUCN SSC Amphibian Specialist Group 2015), and the southern species, *Rhinoderma darwinii*, which is threatened mainly by habitat loss and chytridiomycosis (IUCN SSC Amphibian Specialist Group 2018). The recognition of two distinct species in *Rhinoderma* remains an unresolved debate due to the disappearance of the northern species, *R. rufum*. In this regard, Donoso-Barros (1970) considered that the phenotypic differences do not validate the distinctive status of the two species, but other authors have argued that dissimilarities regarding the distribution, reproductive behavior, and morphology differentiate the two species of the *Rhinoderma* genus (Cei 1958; Formas et al. 1975; Formas 2013).

Very little is known about the acoustic signals produced by these two species of *Rhinoderma.* In 1990, a description of the advertisement calls of both species was published (Penna and Veloso 1990). These authors reported that the calls of *R. darwinii* had a smaller number of notes, lower dominant frequency, and shorter duration of calls and notes relative to those of *R. rufum.* However, this description was based in only four and eight individuals of *R. darwinii* and *R. rufum*, respectively. Audio recordings of these calls are available in Penna (2005). More recently, Serrano et al. (2020) reported a comprehensive analysis of geographical variation of the advertisement calls of *R. darwinii*.

Unsuccessful efforts implementing traditional visual and acoustic surveys have been carried out with the purpose of finding historic populations of the Critically Endangered Northern Darwin's Frog, *R. rufum* (Bourke et al. 2012; Cuevas 2014; Soto-Azat et al. 2013). The last record of the species dates back to 1981, as documented by the capture of four males near the locality of Paredones in Central Chile (34°38'39.6"S 71°54'15.8"W) by Nelson Diaz. These individuals were transported to the

Correspondence. **mpenna@med.uchile.cl* (MP); *jose.rano@gmail.com* (JMS); *gabriel.bidart@ug.uchile.cl* (GBE)

Faculty of Medicine of the University of Chile, where one of us (MP) recorded their release calls at that time. Morphometric data for these specimens are not available. In this manuscript, we describe those recordings of the release calls of *R. rufum*, about 40 years after they were obtained.

Materials and Methods

The release calls were prompted by gently finger-pressing the flanks of the individuals. The soft sounds produced were recorded using an omnidirectional microphone (UHER M517) and a magnetic tape recorder (UHER 4400 Report Stereo IC) under laboratory conditions, at an air temperature of 20 °C. In December 2018, the tapes of the recordings were digitized in WAV format with a sampling rate of 44,100 Hz at 24 bits. As making the acoustic signals of Endangered species available in curated collections of animal sounds is a highly recommended resource, following Toledo et al. (2015), the digitized recordings of the release calls of *R. rufum* were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (CSIC, FZ Sound Code: 12943). To eliminate interference caused by low frequency noise from the original recording, a digital band-stop filter from 0 to 400 Hz was applied to the digital recordings. Three temporal variables were analyzed focusing on the succession of notes typically composing a release call (Köhler et al. 2017), as follows: total number of notes, note rate (number of notes / time from first note onset to last note offset), and note duration. The following spectral variables were also analyzed: for notes in which a harmonic structure was evident, the highest and lowest frequency for the first three harmonic segments (Hz) were measured. In addition, in the notes that lacked harmonics and contained only chaos, the center frequency (i.e., the one having the largest energy content) of the spectrum was measured. Finally, chaos proportion (% of the duration of chaos segments relative to total note duration) was calculated for notes in which this non-linear phenomenon occurred (following Serrano et al. 2020). The acoustic analysis was made using the software Raven Pro 1.4 (Cornell Lab of Ornithology, Bioacoustics Research Program, Ithaca, New York, USA) making manual selections. Spectral parameters were obtained with a fast Fourier transform and a Hanning window of 256 points, the settings used by Köhler et al. (2017). The means and coefficients of variation (CV = 100 × SD/mean) were calculated for all notes recorded from each of the individuals. Oscillograms and spectrograms were obtained using the package Seewave in R (Sueur et al. 2018).

Results and Discussion

The release call of *R. rufum* consists of mid-pitched squeaks repeated in sustained trains at the beginning of the manipulation, and the emission rate decays thereafter to isolated notes, resuming a high rate when the flanks are pressed again during handling. The notes have a mixed harmonic and chaotic composition. The harmonic portions are frequency modulated, having ascending-descending, ascending, or descending patterns, and the dominant frequency usually corresponds to the second harmonic (Fig. 1A). One individual was quite responsive, producing a total of 131 notes, and the other three were less active, producing 70, 13, and 17 notes in total (Table 1). Forty-three percent of recorded calls consisted of

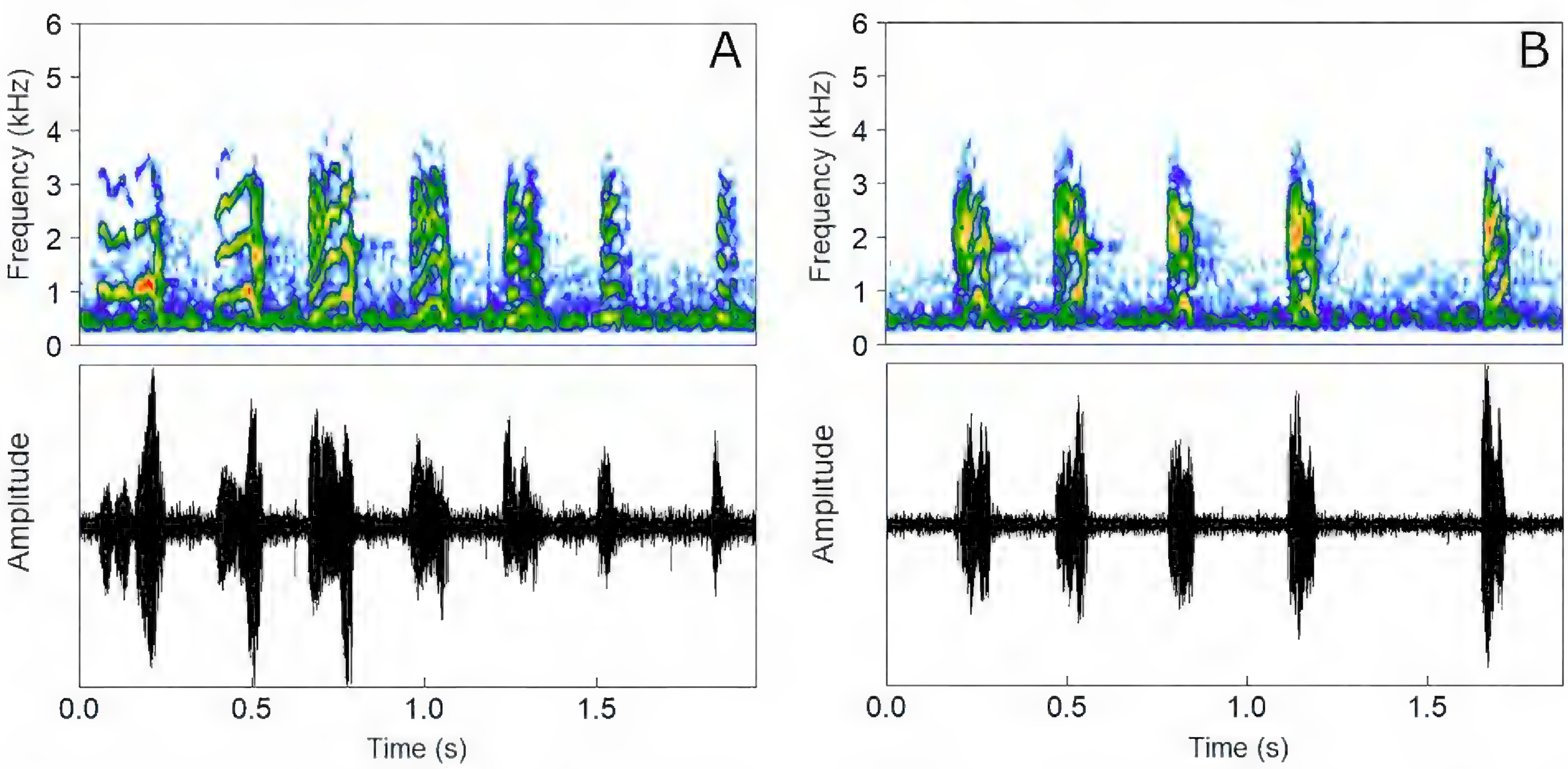

Fig. 1. Spectrograms and oscillograms of multi-note release calls emitted by males of *Rhinoderma rufum*. Two representative calls are shown, one having mainly harmonic structure (**A**, individual 1 in Table 1) and another having mainly chaotic structure (**B**, individual 2 in Table 1). Sample rate: 44.1 kHz, frequency bandwidth: 20 Hz. The oscillograms and spectrograms were obtained using the package Seewave (Sueur et al. 2018).

Table 1. Means, ranges (in parentheses), and coefficients of variation among individuals (CV, expressed as the percentage) for the acoustic characteristics in the release calls of *Rhinoderma rufum*. Center frequency was measured in the chaos components of the notes. Abbreviations: H*f*: highest frequency; L*f*: lowest frequency, of the three first harmonics. CV: coefficient of variation.

Acoustic characteristics	Individuals				Mean	CV
	1	2	3	4		
Number of notes	131	70	13	17		
Number of calls	49	32	8	6		
Notes per call in multi-note calls	3.73 (2–13)	3.11 (2–10)	2.67 (2–3)	3.2 (2–5)	3.4	110.2
Note rate (notes/s) in multi-note calls	4.4 (1.78–7.30)	4.35 (2.35–7.81)	3.84 (2.26–5.09)	4.12 (2.65–5.39)	4.36	32.9
Note duration (s)	0.09 (0.023–0.202)	0.068 (0.020–0.139)	0.081 (0.014–0.135)	0.047 (0.020–0.097)	0.8	52.8
H*f*1 (Hz)	798 (474–1,895)	842 (474–1,292)	873 (560–1,249)	768 (517–1,249)	817	30
L*f*1 (Hz)	611 (474–1,637)	624 (474–947)	689 (517–1,034)	668 (517–1,120)	628	30
H*f*2 (Hz)	1,490 (689–3,661)	1,581 (947–2,412)	1,600 (1,120–2,110)	1,467 (947–2,412)	1,530	31.2
L*f*2 (Hz)	1,163 (646–3,747)	1,227 (732–1,895)	1,327 (991–1,938)	1,299 (991–2,239)	1,213	34.2
H*f*3 (Hz)	2,058 (1,034–3,488)	2,353 (1,335–3,488)	2,498 (1,593–3,661)	2,135 (1,335–3,618)	2,210	29
L*f*3 (Hz)	1,601 (991–2,885)	1,861 (1,120–2,967)	1,961 (1,464–3,144)	1,923 (1,464–3,316)	1,758	27.6
Center frequency (Hz)	1,196 (474–2,067)	1,375 (517–1,938)	1,142 (1,120–1,163)	1,180 (1,120–1,335)	1,238	32.2
Chaos (%)	73 (9–100)	41 (14–100)	30.1 (18–55)	74 (29–100)	68	47.4

single notes. Multiple-note calls had two to 13 notes. The note rate calculated for multiple-note calls ranged from 1.78 to 7.81 notes/s. Note durations measured for all the notes produced by the four individuals ranged between 0.014 and 0.202 s. Segments with harmonic components occurred in 54% of the notes, and 97% of the notes contained non-linear segments identified as chaos phenomena. For the harmonic segments, the frequencies of the first, second, and third harmonics ranged between 474–1,895, 646–3,747, and 991–3,661 Hz, respectively, and the center frequency in notes with non-linear phenomena ranged from 474 to 3,618 Hz. The proportion of chaos segments ranged from 9–100% in the notes that contained these components. All acoustic characteristics measured were highly variable among individuals, the temporal characteristic with the lowest CV was note rate (32.9%), and the number of notes per call had the highest CV (110.2%) (Table 1). The harmonic spectral characteristics had CVs ranging from 27.6 to 34.2% and the non-linear spectral characteristics had CVs of 32.2% and 47.4%.

The large variation in the number of release calls of *R. rufum* and the dynamic character of their acoustic properties suggest that their occurrence is likely influenced by motivation or stress levels of the animals (Blumstein and Chi 2012; Moreno-Gómez et al. 2015). In addition, the high variability of the acoustic properties of these release calls argues against the potential relevance of these signals in individual recognition, as occurs for advertisement calls in other anurans (Feng et al. 2009; Serrano et al. 2020). In the advertisement call of *R. darwinii*, the variation of non-linear components has been reported to be related with the SVL size of these frogs, with chaos being inversely related to the emitter's size (Serrano et al. 2020). However, relationships of this kind could not be evaluated in *R. rufum* due to the low number of individuals recorded and the lack of morphometric measurements for these individuals.

Considering the conservation status of the genus *Rhinoderma* and the discussion regarding the validity of the two recognized species, it is relevant to acknowledge that *R. darwinii* issues release calls in response to tactile stimulation but not consistently. For example, continental populations of this species produce only single-note release calls when being handled (A. Valenzuela-Sánchez, Pers. Comm.), but individuals from the island of Chiloé very rarely produce these kinds of vocalizations. As such, comparing the release calls produced by *R. darwinii* would provide further evidence for behavioral differences between the *Rhinoderma* species. The results of an ongoing study comparing the advertisement calls of both species show that these signals are rather similar, not contributing to the assignment of a specific status to both taxa (G. Bidart-Enríquez et al., In Prep.). This brief contribution is also meant to prompt renewed efforts to rediscover the now long-missing local populations of Darwin's Frog in central Chile. Knowledge of its release calls may also contribute to setting up acoustic monitoring to detect potential populations of this presumably extinct species.

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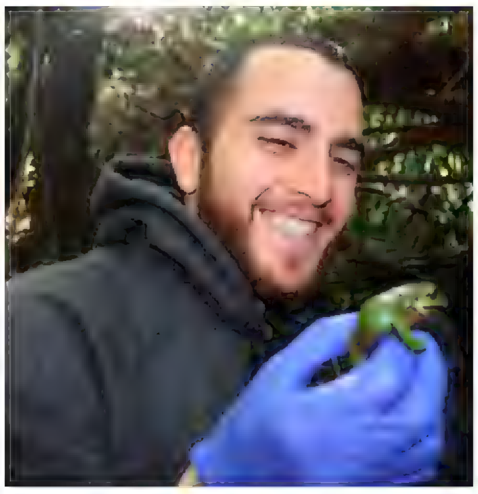
José Manuel Serrano is currently a postdoctoral researcher at Universidad Nacional Autónoma de México (Museo de Zoología Alfonso L. Herrera, Facultad de Ciencias, UNAM) and an associate researcher at the NGO Ranita de Darwin (Santiago, Chile). His interests are related to the acoustic communication, ecology, and conservation of frogs.



Gabriel Bidart-Enriquez earned his Bachelor's degree as an Environmental Biologist at the Universidad de Chile (Santiago, Chile) and is co-founder of the citizen-science project Sappea, a multimedia platform for the recognition and records of Chilean amphibians.

Mario Penna Varela is a Professor in the Programa de Fisiología y Biofísica, Universidad de Chile (Santiago, Chile). His area of general interest is acoustic communication, particularly anuran bioacoustics.